Tetrahydrobiopterin increases insulin sensitivity in patients with type 2 diabetes and coronary heart disease

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Submitted 2 February 2004; accepted in final form 17 July 2004

Nystöm, Thomas, Arne Nygren, and Åke Sjöholm. Tetrahydrobiopterin increases insulin sensitivity in patients with type 2 diabetes and coronary heart disease. Am J Physiol Endocrinol Metab 287: E919–E925, 2004. First published July 20, 2004; doi:10.1152/ajpendo.00046.2004.—Tetrahydrobiopterin (BH4) is an essential cofactor of nitric oxide synthase that improves endothelial function in diabetics, smokers, and patients with hypercholesterolemia. Insulin resistance has been suggested as a contributing factor in the development of endothelial dysfunction via an abnormal pteridine metabolism. We hypothesized that BH4 would restore flow-mediated vasodilation (FMD, endothelial-dependent vasodilation), which may affect insulin resistance in type 2 diabetic patients. Thirty-two subjects (12 type 2 diabetic subjects, 10 matched non-diabetic subjects, and 10 healthy unmatched subjects) underwent infusion of BH4 or saline in a random crossover study. Insulin sensitivity index (SI) was measured by hyperinsulinemic isoglycemic clamp. FMD was measured using ultrasonography. BH4 significantly increased SI in the type 2 diabetics [3.6 ± 0.6 vs. 4.9 ± 0.7 × 10⁻⁴ dl·kg⁻¹·min⁻¹/μU·ml⁻¹, P < 0.05], while having no effects in nondiabetics [8.9 ± 1.1 vs. 9.0 ± 0.9 × 10⁻⁴ dl·kg⁻¹·min⁻¹/μU·ml⁻¹, P = 0.92] or in healthy subjects [17.5 ± 1.6 vs. 18 ± 1.8 × 10⁻⁴ dl·kg⁻¹·min⁻¹/μU·ml⁻¹, P = 0.87]. BH4 did not affect the relative changes in brachial artery diameter from baseline FMD (%) in type 2 diabetic subjects (2.3 ± 0.8 vs. 1.8 ± 1.6%, P = 0.42), nondiabetic subjects (5.3 ± 1.1 vs. 6.6 ± 0.9%, P = 0.32), or healthy subjects (11.9 ± 0.6 vs. 11.0 ± 1.0%, P = 0.48). In conclusion, BH4 significantly increases insulin sensitivity in type 2 diabetic patients without any discernible improvement in endothelial function.


diabetics have a worse outcome after a myocardial infarction compared with nondiabetics, the mortality in this disease being elevated three- to fivefold compared with nondiabetics (39). A hallmark of diabetic vascular disease is endothelial dysfunction, one of the earliest events identified in the pathogenesis of atherosclerosis (3). Hyperglycemia and insulin resistance are the main metabolic characteristics of type 2 diabetes (17). Their negative influence on vascular endothelial function may explain the diabetic angiopathy.

Insulin increases glucose disposal in skeletal muscle by recruitment and activation of specific glucose transporter proteins (27). Insulin also activates endothelial nitric oxide synthase (eNOS) in endothelial cells via the classical insulin pathway (38). An important physiological mechanism to amplify insulin’s overall action to increase glucose disposal may be by augmenting the delivery of insulin and glucose via capillary recruitment (2, 8). Impaired endothelium-dependent vasodilation is associated with insulin resistance, and this association may be represented in the vasculature by abnormalities in insulin-stimulated endothelial function. There is evidence that insulin-mediated skeletal muscle vasodilation is nitric oxide (NO) dependent in humans (28). These effects are dependent on glyceremia and diminished in patients with obesity-associated insulin resistance or type 2 diabetes (11, 18, 29).

Interest engendered the possibility that tetrahydrobiopterin (BH4), a critical cofactor for eNOS, may be deficient in various conditions associated with impaired endothelial function (15, 25). Conversely, treatment with BH4 has been shown to augment endothelium-dependent vasodilation in humans with hypercholesterolemia and diabetes and in smokers (13, 14, 30, 31). In the setting of oxidative stress by hyperglycemia, BH4 depletion is seen, causing an uncoupling of the /-arginine-NO pathway, and this results in increased formation of oxygen radicals, i.e., peroxynitrite and superoxide anion (O2·-) (25, 32, 37). In addition, BH4 depletion has been shown to decrease NO production (32). However, the influence of BH4 on insulin resistance in patients with type 2 diabetes has not been evaluated.

The purpose of our study was to determine whether BH4 improves flow-mediated endothelial function and insulin sensitivity in type 2 diabetic subjects.

MATERIALS AND METHODS

The study population consisted of 32 male Caucasian individuals, of whom 12 were type 2 diabetic subjects, 10 were matched nondiabetic subjects, and the remainder an unmatched group of ten healthy subjects (Table 1). Type 2 diabetic subjects were matched by age and body mass index, with a group of nondiabetic subjects. All subjects (except healthy subjects) had an established, stable coronary artery disease (CAD), having suffered a myocardial infarction (MI) 3–6 mo before the onset of the study procedure. The study was approved by the local ethics committee, and written informed consent from the patients was obtained.

Type 2 diabetic subjects. Twelve middle-aged, moderately obese male patients with mild type 2 diabetes of short duration and with an established CAD volunteered for the study. None of the patients showed signs of microvascular diabetic complications such as overt nephropathy, neuropathy, or retinopathy. However, they had all suffered an MI. All pharmacological treatments are given in Table 1. Glycemic control was achieved by diet alone (in one patient), diet plus oral hypoglycemic agents (metformin, sulfonylurea, or both), insulin alone, or insulin plus oral hypoglycemic agents.

Nondiabetic subjects. This group was well matched with the diabetic group (Table 1). They had all suffered an MI. All subjects were tested twice, with at least a 1-wk interval, showing fasting blood glucose levels of 4.8 ± 0.2 and 4.9 ± 0.1 mmol/l, respectively. All pharmacological treatments are given in Table 1.

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that the reduced form of BH4 was infused, the investigator thus study design was that BH4 is highly reactive in room air. To ensure washout period for 1 wk. The rationale for using a single-blinded single-blind (blinded for patient), cross-over random order and with a

![Fig. 1. After a 12-h overnight fast, subjects underwent infusion of BH4, di

Table 1. Baseline clinical and biochemical characteristics of the study groups

<table>
<thead>
<tr>
<th>Subjects</th>
<th>T2DM (n = 12)</th>
<th>Non-DM (n = 10)</th>
<th>Healthy Subjects (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59 ± 2</td>
<td>57 ± 2</td>
<td>29 ± 4</td>
</tr>
<tr>
<td>Duration of diabetes, yr</td>
<td>5.1 ± 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.6 ± 1.1</td>
<td>28.2 ± 1.2</td>
<td>24.3 ± 0.6</td>
</tr>
<tr>
<td>Smoker, yes/no</td>
<td>1/1</td>
<td>1/9</td>
<td></td>
</tr>
<tr>
<td>sBP, mmHg</td>
<td>122 ± 26</td>
<td>120 ± 22</td>
<td>111 ± 1</td>
</tr>
<tr>
<td>dBP, mmHg</td>
<td>80 ± 3</td>
<td>78 ± 2</td>
<td>71 ± 2</td>
</tr>
<tr>
<td>Urinary protein, mg/l</td>
<td>58 ± 23</td>
<td>8 ± 2</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

Patients treated with

Insulin, yes/no 7/5
Insulin dose, units/day 39 ± 5
Sulfonylurea, yes/no 5/7
Biguanide, yes/no 1/11
ACE inhibitor/ARB, yes/no 8/4 4/6
β-Blocker, yes/no 12/0 10/0
Calcium flow inhibitor, yes/no 1/1 1/9
Acetylsalicylic acid, yes/no 12/0 10/0
Statins, yes/no 9/3 7/3

Serum Hb A1c, % 6.1 ± 0.2 4.8 ± 0.1† 4.6 ± 0.1
Serum creatinine, μmol/l 88 ± 2.0 83 ± 4 84 ± 2
Serum total cholesterol, mmol/l 4.1 ± 0.1 4.6 ± 0.2 3.8 ± 0.3
Serum HDL-cholesterol, mmol/l 1.0 ± 0.1 1.2 ± 0.1* 1.3 ± 0.1
Serum LDL-cholesterol, mmol/l 2.4 ± 0.1 2.8 ± 0.2 2.0 ± 0.2
Serum triglycerides, mmol/l 1.6 ± 0.1 1.4 ± 0.1 1.1 ± 0.2
Serum folate, mmol/l 9.8 ± 0.9 10.7 ± 2.3 11.9 ± 3.0
Serum vitamin B12, μmol/l 303 ± 20 305 ± 24 274 ± 24

Data are presented as means ± SE. T2DM, type 2 diabetic subjects; Non-DM, nondiabetic subjects; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; sBP, systolic blood pressure; dBP, diastolic blood pressure. *P < 0.05, †P < 0.01 compared with T2DM.

Healthy subjects. No one was suffering from any disease or taking any medication. Questionnaires did not reveal any family history of diabetes or cardiovascular disease. No one was using tobacco products.

Study protocol. A schema of the experimental design is outlined in Fig. 1. After a 12-h overnight fast, subjects underwent infusion of BH4 (500 μg/min; Schircks Laboratories, Jona, Switzerland), in accord with other studies (13, 14, 30), or saline (0.9% NaCl; Baxter) in a single-blind (blinded for patient), cross-over random order and with a washout period for 1 wk. The rationale for using a single-blinded study design was that BH4 is highly reactive in room air. To ensure that the reduced form of BH4 was infused, the investigator thus prepared BH4 immediately before infusing the substance. Calculations of blood flow, changes in arterial vasodilation, and clamp data were done unaware of the subjects or the procedure. Subjects were taking their medicines as usual between the test periods. Subjects were not allowed to eat or drink anything but water, and they refrained from their medicines on the morning of the test day. Insulin was not given after 6:00 PM the day before the test. BH4 was infused 15 min after priming for the hyperinsulinemic clamp and throughout the clamp, thus for a total of 105 min (Fig. 1). The sensitivity to insulin-mediated glucose disposal was measured with a hyperinsulinemic isoglycemic clamp technique. Subjects were clamped with regard to their fasting blood glucose levels. If glucose levels differed between treatments (saline vs. BH4), correction was done by prepriming with either insulin or glucose infusion to maintain exactly the same blood glucose level as for the week before. One reason for use of isoglycemic clamp instead of euglycemic clamp is that one avoids artifacts caused by acute lowering of glucose levels with insulin. The brachial artery response to reactive hyperemia was measured with ultrasonogram at onset and at 100 min in the steady-state clamp procedure. Ultrasonogram measures flow, which, in turn, may represent endothelial func-

Fig. 1. Experimental design. Before any infusions, subjects underwent flow-mediated vasodilation (FMD) and nitroglycerine-mediated vasodilation (NTG) measurements with ultrasonogram. Hyperinsulinemic isoglycemic clamps for a total of 120 min were undertaken in single-blind crossover studies with saline or tetrahydrobiopterin (BH4, 500 μg/min). At 105 min, subjects were reexamined with ultrasonogram for FMD and NTG responses. See text for details.

tion. Thus, at onset, every patient was examined twice with regard to flow-mediated vasodilation (FMD) and nitroglycerine-mediated vasodilation (NTG) without insulin, saline, or BH4 infusions. Thereafter, every patient was examined with or without BH4 at steady-state insulin clamp (Fig 1).

Brachial artery flow and diameter. The diameter of the target artery was measured from two-dimensional ultrasound images, using a 7.0 MHz linear array transducer and a standard 128XP/10 system (Accuson, Mountain View, CA) according to Celermajer et al. (5). After a 60-min resting time, the subject’s left arm was immobilized, and the transducer was fixed in the same position throughout the study with the assistance of a mechanical arm. The brachial artery was scanned longitudinally, and the transmit (focus) zone was set to optimize images of the lumen arterial wall interface. The B-mode images were magnified by a resolution box and obtained with gating from the R wave of the electrocardiogram as trigging mode. The condition of reactive hyperemia was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 300 mmHg for 4.5 min, followed by release [endothelial-dependent vasodilation (FMD)]. Measurements were made at baseline (after 30 min of supine rest) and at 45 and 60 s after cuff release. Then, after a 10-min rest, 0.4 mg of nitroglycerine spray was applied, and new images were obtained 4 min later [endothelial-independent vasodilation (NTG)]. The relative changes in vessel diameter are expressed as percentages after reactive hyperemia (FMD%) and after nitroglycerine administration (NTG%) from baseline scan. Brachial artery diameter, the means of two images, was measured with an automated computerized analyzing system, according to the sonomorphological definition of Wendelhag et al. (36). In brief, this analyzing program is a PC/Windows-based software with digitized ultrasound image. The starting point of the measurement area is set by the operator, and a 10-mm box is automatically drawn. The different echo interfaces are automatically outlined. If obvious errors are detected, it is possible to modify the measurement by marking a correct echo in the ultrasound image. In this case, only one or two manually marked points are needed to guide the automatic system to the correct interface. This automated system has proved superior to manual measurements, with a dramatically improved reproducibility both in inter- and intraobserver coefficient of variations (36).

Arterial blood flow velocity rates were obtained using a pulsed Doppler signal at 70° angles to the vessel in the center of the artery. Ultrasound images were made 15 s after cuff release with the freeze mode. The volume flow was calculated by multiplying the velocity time integral of the Doppler flow signal for the mean of three pulse waves by the heart rate and vessel cross-sectional area. Calculations of blood flow and changes in arterial vasodilation were done unaware of the subjects or the procedure.

The coefficients of repeatability and variance were determined in all subjects between first and second visit at basal condition. The mean (SE; range) for FMD% was 5.7 (1.3; 3.9–7.6) at the first visit and 6.3 (1.2; 4.5–8.0) at the second visit. The mean (SE; range) for NTG% was 17.7 (1.0; 15.7–19.7) at the first visit and 15.5 (0.8; 13.7–17.2) at the second visit. Correlation (r) between the first and second visit was r = 0.73 for FMD% and r = 0.59 for NTG%. Repeatability coeffi-
Hyperinsulinemic isoglycemic clamp. Hyperinsulinemic clamps were performed according to DeFronzo et al. (9). In brief, a superficial dorsal hand vein was cannulated in retrograde fashion with a 21-gauge butterfly needle and kept patent by a slow infusion of saline solution. The hand was kept warm by an electric device for intermittent sampling of arterialized venous blood. After that, one intravenous catheter was inserted into the left antecubital vein for substrate (insulin/glucose) and drug infusion (BH4/saline). During the 120 min of the test, insulin (Human Actrapid, 40 mU·m−2·min−1; Novo Nordisk) was infused along with 20% dextrose (Fresenius Kabi). The rate of dextrose infusion was adjusted to achieve a blood glucose level compared with subjects’ fasting glucose levels, on the basis of arterialized samples withdrawn every 5 min from the dorsal hand vein catheter (heated-air box at 55°C, University of Nottingham Department of Physiology and Pharmacology). The glucose clamp-derived index of insulin sensitivity [SI; 10−4 ·dl·kg−1·min−1·(µU/ml)] was calculated from the glucose infusion rate (GIR), corrected for body weight, during the final 30 min as follows: \( SI = \frac{GIR_{SS}}{G_{SS} \times DI_{SS}} \), where GIRSS is the steady-state GIR (mg/min), GSS is the steady-state blood glucose concentration (mg/dl), and DISS is the difference between basal and steady-state plasma insulin concentrations (µU/ml). This calculation is assumed to correct for differences in prevailing glucose and insulin concentrations.

Analytical methods. Blood glucose levels were determined by the glucose oxidase method with a glucose analyzer (YSI 2300, STAT VETI PLUS). All plasma samples were drawn from the dorsal hand vein catheter, anticoagulated with EDTA, centrifuged for ~15 min, and then stored at −20°C pending analysis. Plasma levels for each subject were run in the same assay to eliminate interassay variations. Insulin and C-peptide levels were measured using enzyme-linked immunosorbent assays (Pharmacia and Mercodia, both Uppsala, Sweden).

Statistical analysis. Results are shown as means ± SE. Comparisons between groups, treatments, and time were made by a two-way analysis of variance (ANOVA) for repeated measures. Furthermore, using a sequence factor (regarding the order in which subjects were examined) in the ANOVA model, we took care of any carry-over effects. Significant differences by ANOVA were followed by post hoc Sheffe’s test. For baseline clinical and biochemical characteristics, data were compared by unpaired two-tailed Student’s t-test analysis. \( P < 0.05 \) was deemed statistically significant.

RESULTS

Clinical and biochemical baseline characteristics. All data are given in Table 1. Comparisons of groups were made only between the matched groups (not for the unmatched healthy subjects). There were significant differences between type 2 diabetic subjects compared with nondiabetic subjects in levels of total cholesterol, HDL-cholesterol, and Hb A1C; otherwise, no significant differences were noted between the matched groups.

Hyperinsulinemic isoglycemic clamp data. All clamp data are given in Table 2. Blood glucose, plasma insulin, and plasma C-peptide levels were similar between saline and BH4 infusions in type 2 diabetic subjects as well as in nondiabetic subjects and in healthy subjects (Table 2). BH4 significantly increased GIR in the type 2 diabetics (3.3 ± 0.3 vs. 4.4 ± 0.4 mg·kg−1·min−1, \( P < 0.05 \)) while having no effects in non-

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Table 2. Data during hyperinsulinemic clamp with saline vs. BH4 in T2DM, non-DM, and healthy subjects

<table>
<thead>
<tr>
<th>T2DM (n = 12)</th>
<th>Blood glucose, mmol/l</th>
<th>Plasma insulin, pmol/l</th>
<th>Plasma C-peptide, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7.0 ± 0.4</td>
<td>6.8 ± 0.3</td>
<td>0.77 ± 0.1</td>
</tr>
<tr>
<td>BH4</td>
<td>6.9 ± 0.4</td>
<td>6.8 ± 0.4</td>
<td>0.68 ± 0.1</td>
</tr>
<tr>
<td>Non-DM (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4.9 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>0.93 ± 0.2</td>
</tr>
<tr>
<td>BH4</td>
<td>4.7 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Healthy subjects (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4.6 ± 0.1</td>
<td>5.6 ± 0.1</td>
<td>0.74 ± 0.1</td>
</tr>
<tr>
<td>BH4</td>
<td>4.7 ± 0.1</td>
<td>5.7 ± 0.1</td>
<td>0.70 ± 0.1</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. BH4, tetrahydrobiopterin.
diabetics (5.5 ± 0.6 vs. 5.2 ± 0.5 mg·kg\(^{-1}\)·min\(^{-1}\), P = 0.83) or in healthy subjects (11.9 ± 1.0 vs. 12.4 ± 0.8 mg·kg\(^{-1}\)·min\(^{-1}\), P = 0.91). After correcting for the assumed different glucose and insulin concentrations between the procedures, using S\(_0\), we were still able to show a significant improvement from BH\(_4\) in type 2 diabetic subjects (Fig. 2). Also, large differences in GIR were seen between groups: diabetic subjects compared with nondiabetic subjects (3.3 ± 0.3 vs. 5.5 ± 0.6 mg·kg\(^{-1}\)·min\(^{-1}\), P < 0.01) as well as nondiabetic subjects compared with healthy subjects (5.5 ± 0.6 vs. 11.9 ± 1.9 mg·kg\(^{-1}\)·min\(^{-1}\), P < 0.001).

**Endothelial-dependent vasodilation.** All flow-mediated data are given in Table 3. Baseline brachial artery diameter was similar between saline and BH\(_4\) infusions at onset and steady-state insulin clamp procedure in all groups (Table 3). However, at onset without any infusions, brachial artery diameter in healthy subjects was significantly smaller compared with type 2 diabetic subjects (Table 3). At steady-state insulin clamp period, BH\(_4\) infusion did not affect FMD% in type 2 diabetic subjects, nondiabetic subjects, or healthy subjects compared with saline infusion (Fig. 3A). In contrast with the modest relative responses in FMD noted in type 2 diabetic subjects, much larger responses were noted in nondiabetic subjects. Thus there was a significant difference in FMD%, at onset without any infusions, between type 2 diabetic subjects and nondiabetic subjects (1.6 ± 0.5 vs. 5.7 ± 0.7%, P < 0.01) as well as between nondiabetic subjects and healthy subjects (5.7 ± 0.7 vs. 11.4 ± 1.5%, P < 0.001). These differences between groups in vascular reactivity were mirrored by corresponding differences in S\(_0\), with a very modest reaction in type 2 diabetic subjects compared with nondiabetic subjects and healthy subjects (Fig. 2).

**Endothelial-independent vasodilation.** All nitroglycerin-mediated data are given in Table 3. Baseline brachial artery diameter was similar between saline and BH\(_4\) infusions at onset and steady-state insulin clamp procedure in all groups (Table 3). However, brachial artery diameter in healthy subjects was significantly smaller compared with type 2 diabetic subjects and nondiabetic subjects (Table 3). The relative increases in brachial artery diameter elicited by nitroglycerin did not change between saline and BH\(_4\) infusions at steady-state insulin clamp in any group (Table 3). At onset, before insulin clamp, without any infusions, NTG% were decreased in type 2 diabetic subjects compared with healthy subjects (Fig. 3B). Compared with nondiabetic subjects, this decrease in NTG% was borderline significant (Fig. 3B; P = 0.06).

**Brachial artery flow data.** All brachial artery flow data are given in Table 3. At insulin clamp steady state, before cuff inflation, basal artery flow did not differ between saline and BH\(_4\) infusions in type 2 diabetic subjects (50 ± 5 vs. 47 ± 5 ml/min, P = 0.63), nondiabetic subjects (43 ± 5 vs. 42 ± 5 ml/min, P = 0.89), and healthy subjects (35 ± 2 vs. 33 ± 5 ml/min, P = 0.91). However, healthy subjects had a significantly lower basal artery flow compared with both the type 2 diabetes group and the nondiabetes group (Table 3). At insulin clamp steady state, immediately after cuff deflation, the increase in blood flow did not differ between saline and BH\(_4\) infusions in type 2 diabetic subjects (144 ± 20 vs. 148 ± 17 ml/min, P = 0.87), nondiabetic subjects (165 ± 11 vs. 160 ± 14 ml/min, P = 0.77), or healthy subjects (164 ± 15 vs. 150 ± 26 ml/min, P = 0.65). Although not significant, the postischemic shear stress stimulus seemed less in type 2 diabetes subjects (Table 3). However, in this crossover study design, baseline brachial artery diameter and baseline artery blood flow as well as postischemic maximal blood flow did not differ within groups.

### Table 3. Brachial artery data at onset and during steady-state insulin clamp with BH\(_4\) vs. saline in all groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>T2DM Onset</th>
<th>BH(_4) Onset</th>
<th>T2DM Clamp</th>
<th>BH(_4) Clamp</th>
<th>T2DM Non-DM Onset</th>
<th>BH(_4) Non-DM Onset</th>
<th>T2DM Healthy Onset</th>
<th>BH(_4) Healthy Onset</th>
<th>T2DM Healthy Clamp</th>
<th>BH(_4) Healthy Clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline diameter FMD, mm</td>
<td>4.4±0.1</td>
<td>4.7±0.2</td>
<td>4.3±0.1</td>
<td>4.7±0.1</td>
<td>4.2±0.2</td>
<td>4.3±0.2</td>
<td>4.2±0.1</td>
<td>4.4±0.1</td>
<td>3.8±0.1</td>
<td>3.9±0.1</td>
</tr>
<tr>
<td>Maximal diameter FMD, mm</td>
<td>4.5±0.1</td>
<td>4.8±0.2</td>
<td>4.4±0.1</td>
<td>4.8±0.1</td>
<td>4.4±0.2</td>
<td>4.5±0.2</td>
<td>4.5±0.2</td>
<td>4.7±0.1</td>
<td>4.2±0.2</td>
<td>4.2±0.1</td>
</tr>
<tr>
<td>Baseline diameter NTG, mm</td>
<td>4.5±0.1</td>
<td>4.7±0.2</td>
<td>4.3±0.1</td>
<td>4.5±0.1</td>
<td>4.2±0.1</td>
<td>4.3±0.2</td>
<td>4.1±0.2</td>
<td>4.4±0.2</td>
<td>3.9±0.2</td>
<td>3.9±0.1</td>
</tr>
<tr>
<td>Maximal diameter NTG, mm</td>
<td>5.1±0.1</td>
<td>5.3±0.2</td>
<td>5.0±0.1</td>
<td>5.1±0.1</td>
<td>5.0±0.2</td>
<td>5.0±0.2</td>
<td>4.9±0.2</td>
<td>5.1±0.2</td>
<td>4.6±0.2</td>
<td>4.6±0.2</td>
</tr>
<tr>
<td>Baseline flow FMD, ml/min</td>
<td>44±5</td>
<td>50±5</td>
<td>42±3</td>
<td>47±8</td>
<td>50±8</td>
<td>44±5</td>
<td>45±2</td>
<td>42±5</td>
<td>32±2*</td>
<td>35±2*</td>
</tr>
<tr>
<td>Maximal flow FMD, ml/min</td>
<td>130±13</td>
<td>144±20</td>
<td>144±18</td>
<td>148±17</td>
<td>148±18</td>
<td>165±11</td>
<td>154±18</td>
<td>160±14</td>
<td>156±18</td>
<td>165±15</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>57±2</td>
<td>55±2</td>
<td>56±2</td>
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<td>56±2</td>
<td>55±2</td>
<td>57±3</td>
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<td>52±3*</td>
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</tr>
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<td>SBP, mmHg</td>
<td>124±5</td>
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<td>76±2</td>
<td>77±2</td>
<td>71±3*</td>
<td>69±2*</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE, 2-way ANOVA with repeated measurement. See text for details. Onset, without any procedure or infusions; Clamp, steady-state insulin clamp interval 90–120 min; FMD, flow-mediated vasodilation; NTG, nitroglycerin-mediated vasodilation. *P < 0.05 compared with non-DM, †P < 0.05 compared with T2DM, at given time point.

**AJP-Endocrinol Metab • VOL 287 • NOVEMBER 2004 • www.ajpendo.org**
Subjects, significantly better FMD response compared with type 2 atherosclerosis, and diabetes show a strong correlation to shown in the United Kingdom Prospective Diabetes Study.

In consideration of our patients being relatively old and suffering from severe CAD, we were not surprised to obtain this modest FMD in type 2 diabetic subjects because age, atherosclerosis, and diabetes show a strong correlation to endothelial function. In contrast, non-diabetic subjects had a significantly better FMD response compared with type 2 diabetic subjects, and this difference was paralleled by a corresponding difference in insulin sensitivity. Additionally, there was a decrease in NTG response in type 2 diabetic subjects compared with healthy subjects, with a borderline significance between non-diabetic subjects. This finding is consistent with other reports that documented an impaired endothelial-dependent as well as endothelial-independent vasodilation response in type 2 diabetic patients (1, 20).

Baseline artery blood flow was lower in healthy subjects compared with other groups. This could be explained by a smaller brachial artery diameter in the same group. Type 2 diabetic patients tended to have, although not significant, a greater brachial artery diameter with a concomitant decline in the maximal blood flow after postischemic shear stress stimulus compared with the other groups. This may suggest that postischemic shear stress stimulus was different between groups. However, this non-significant difference in postischemic shear stress stimulus can hardly explain the robust differences seen in FMD responses between groups.

Our failure to detect any improvement in endothelial function during BH4 infusion is not consistent with other reports (13, 14, 30, 31). Several potential reasons for this should be considered. One reason may be a matter of the method chosen. Although endothelial dysfunction can be reliably detected noninvasively using high-resolution ultrasonography, investigators demonstrating benefits from BH4 infusion on endothelial dysfunction have mainly used plethysmography (13, 14, 30). However, in one study, endothelial function was measured with ultrasonography during simultaneous infusion of BH4. Subjects from that study were healthy young smokers (31), making it difficult to compare this study with our study. Plethysmography is more often used than ultrasonography when pharmacologically induced blood flow changes are measured. Lind et al. (19) evaluated the relationship between these techniques. They were unable to demonstrate any correlation between these methods in endothelial-dependent vasodilation, clearly indicating that these methods are not compatible. Also, our study design makes comparisons with other studies difficult because we were performing the hyperinsulinemic clamps during BH4 infusion. The reason for this study design was that we wanted to investigate the relationship between endothelial function and insulin resistance in parallel.

Insulin, given intravenously, causes vasodilation in normal subjects, but this response is diminished in insulin resistance and obesity and in patients with type 2 diabetes (10, 18). A number of studies have used total blood flow rates as a measure of insulin’s vascular action, but this approach may mask a significant vascular effect of insulin. There are reports indicating that this effect occurs at least 60 min before any changes in total muscle blood flow (34). Also notable is that microvascular flow rates as a measure of GIR and not total muscle blood flow (6). What may be more important is the distribution of microvascular flow within the muscle. It has been proposed that two blood flow routes occur in muscle: one in contact with myocytes, i.e., nutritive blood flow, able to exchange nutrients and hormones; and one with essentially no contact with myocytes, i.e., nonnutritive blood flow (7). Even though we did not measure microvascular blood flow in this study, one plausible explanation of why the observed enhancement in glucose disposal evoked by BH4 was not paralleled by a corresponding increase in FMD and brachial artery blood flow would thus be that capillary recruitment may have occurred in response to BH4. If this is the case, and total blood flow does not change, then it also follows that blood flow is decreased in other capillaries, i.e., nonnutritive capillaries. Therefore, the observed enhancement in glucose disposal evoked by BH4 may reflect an enhanced effect of insulin upon arterioles, changing
BH₄ promotes glucose disposal in type 2 diabetes

route from nonnutritive to nutritive blood flow, which may have occurred without any changes in FMD of brachial artery. According to the contemporary conceptual framework, insulin stimulates BH₄ synthesis via activation of GTP cyclohydrolase-I, whereas in insulin-resistant states these effects are impaired (33, 35). The oxidative stress associated with insulin resistance may influence endothelial cell function through a depletion of BH₄ because the biosynthesis of BH₄ depends on a normal cellular redox state (16, 25). Supplementation of BH₄ significantly increases the vascular content of BH₄ and restores NO production in aortas from fructose-fed rats and in mesangial cells cultured in high glucose (24, 26). In the current working model, prolonged hyperglycemia in diabetic subjects may result in an alternative metabolism of glucose, e.g., through the polyol pathway, which shifts the cytosolic NADH-to-NAD⁺ ratio toward an oxidative milieu. This altered redox ratio may influence the availability of BH₄ and uncouple eNOS, resulting in an increase in O₂ - production rather than NO. Therefore, BH₄ may have restored an uncoupled state of the L-arginine-NO pathway in our patients, yielding NO instead of O₂ - , thus alleviating insulin resistance via insulin-mediated capillary recruitment. It appears in our study that BH₄ improves insulin resistance only in the setting of hyperglycemia, which may support the hypothesis above. The modest, but significant, effects in glucose disposal by the BH₄ infusion are, in our opinion, of relevance in the pathogenetic rather than in the physiological or therapeutic context. The quantitative effects on insulin sensitivity of other agents tested, e.g., reduced glutathione and L-arginine, range between 7 and 30% (11, 21–23).

It should be noted that BH₄ also serves as a coenzyme for the aromatic amino acid hydroxylases phenylalanine, tyrosine, and tryptophan independently of NO. Besides phenylketonuria, interest has focused on the impact of experimentally induced diabetes on the expression of rat liver phenylalanine hydroxylase with tetrahydrobiopterin and GTP concentrations. Int J Biochem Cell Biol 30: 1047–1054, 1998.


ACKNOWLEDGMENTS

We thank Lotta Larsson and Christina Hall for excellent technical assistance.

GRANTS

Financial support was received from the Swedish Medical Research Council (nos. 72X-12550, 72X-14507, and 72P-14787), GlaxoSmithKline Pharma AB, Petrus and Augusta Hedlund’s Foundation, the Nutricia Research Foundation, the European Foundation for the Study of Diabetes, the Swedish Society of Medicine, the Sigurd and Elsa Golje Memorial Foundation, Svenska Försäkringsföreningen, the Novo Nordisk Foundation, Swedish Match, Sven-

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